Antioxidants and Antityrosinase Activity of Ethanolic Basil Leaves Extract (Ocimum americanum L.) and Eugenol

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ABSTRACT

Harm effect from UV light, both UV-A and UV-B is contributing as a disease trigger and has an impact on human health. To investigate the bioactive compound, antioxidant, and the potential of basil leaves as antiaging sources particularly as the tyrosinase inhibitor. This study used phytochemical screening for the bioactive compound, DPPH scavenging activity for antioxidant assay, and tyrosinase inhibition activity for the antiaging property. The phytochemical screening shows that the basil leaves extract has flavonoid, saponin, phenol, steroid, and alkaloid. The basil leaves extract has lower antioxidant activity (20.55 ± 0.04 μg/mL) compared with eugenol (2.44 ± 0.26 μg/mL) through DPPH scavenging activity. The basil leaves extract (35.59 ± 0.83 μg/mL) has lower antiaging activity particularly as antityrosinase activity compared with eugenol (10.87 ± 0.41 μg/mL). Our findings suggest that basil leaves can be used as an antioxidant and antiaging source, particularly as a tyrosinase inhibitor.

Keywords: Antioxidant; Tyrosinase; Basil; Eugenol; Antiaging

INTRODUCTION

Sunray is an electromagnetic wave that is a source of all kinds of rays. Sunray on the Earth surface is composed of several spectra, i.e. infrared light (> 760 nm), visible light (400-760 nm), ultraviolet light A (UVA) (315-400 nm), UVB light (290-315 nm), and UVC light (100-290 nm) which is very dangerous, have energy very high and are carcinogenic. But nowadays, the thinning of the ozone layer opens opportunities for various diseases and health disorders (D’Orazio et al., 2013). It is known from various studies that there is a variety of fruits and vegetables containing antioxidants and it is believed can be a lightening natural ingredient of the skin because it has the effect of antityrosinase (antihyperpigmentation) or prevent excessive pigment production. One of them is basil leaves (Ocimum americanum L.), which contain phenolic compounds and flavonoids that can serve as antityrosinase that can inhibit the tyrosinase enzyme as a pigment-producing enzyme. In general, the basil leaves are medium-sized plants and herbs which have a high 3-5 cm and have a flower with the size 8-12 mm can be white, pink, or purple (Khair-ul-Bariyah, Ahmed and Ikram, 2012). Basil is an angiosperm that has the seeds and structures of its cell walls rigid and composed of cellulose (Girsang et al., 2015).

The importance of the economic conditions and the spread of basil leaves globally, in both its use as an ingredient or traditional medicine, realized the importance of investigating in terms of pharmacological and toxicologic to see the efficacy and safety of consumption of basil leaves (Güez et al., 2017). According to research conducted by Rasul et al., 2011, that is by using topical cream extract 3% of basil leaves are applied to the skin of the cheeks as control of erythema and melanin skin on 11 voluntary humans with a period of 12 weeks, and the results obtained in the presence of anterythema of the leaf extracts of the basil. According to research conducted by Yonathan, Lintong and Durry, 2016, found in their research believed a compound of flavonoids contained in cocoa beans is believed to be used as an antihyperpigmentation. There is a decrease in the average value of the skin’s melanin pigment before and after exposure to sunlight due to the administration of extracts. Based on the problems and the research that supports it and remember it is used less in the medical field, making researchers want to find out what bioactive compound, antioxidant effect, and the potential of basil leaves (Ocimum americanum L.) as antiaging sources particularly as the tyrosinase inhibitor.

METHODOLOGY

Materials

The materials used on the phytochemical screening is Basil leaves extract, Ethanol 70%, Aquades, 2,2 Diphenyl-1-picrylhydrazyl (Sigma D9132), Methanol absolute (Merck 106092500), DMSO (Merck 1029522500), FeCl3 (1% in ddH2O) (Merck 103943), aquabidest (ddH2O), Glacial acetic acid 100% (Merck 1000630510), H2SO4

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DOI: 10.22146/mot.56081 | Traditional Medicine Journal, 26(2), 2021
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Methods

Preparation of Basil Leaves Extract (BLE)

Fresh basil leaves (Ocimum americanum L.) were obtained from the Cigugur Girang Village, Parongpong District, Bandung Regency. The plants were identified by herbarium staff, Department of Biology, School of Biological Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The fresh basil leaves were wet sorted then it was washed and dried using a food dehydrator and then into simplicia mashed to obtain simplicia basil leaves powder.

% Drying Shrinkage = \frac{\text{weight of the dried simplicia}}{\text{weight of the fresh basil leaves}} \times 100%

Preparation of Ocimum americanum L. extract

This procedure was carried out by extraction using the technique of maceration using ethanol solvent 70%, the filtrate was accommodated every 24 hours until the filtrate turned colorless then ethanol filtrate 70% evaporated until ethanol 70% extract shaped paste was obtained.

Phytochemical Screening

In this research, phytochemical screening had been conducted to determine the bioactive component found in extracts of basil leaves (Ocimum americanum L.). Identification of the chemical contents of the screening consists of alkaloids, steroidal/terpenoids, saponins, flavonoids, tannins, phenols, and terpenoids (Widowati et al., 2016, 2017, 2018).

Flavonoids Test

The sample of basil leaves extracts as much as 10 mg dissolved in 2 N HCl in the reaction tube. Then added Mg/Zn and heated for 5-10 minutes, then cooled and strain and added amil alcohol as much as 1 ml. If extracts produce a red/orange color, the sample contains a flavonoid compound (Widowati et al., 2016, 2017, 2018).

Alkaloids Test

Sample of basil leaves as much as 10 mg dissolved in aquabidest as much as 5 ml was dried in the water bath. The resulting residue was then dissolved with 5 ml 2 N HCl. The acquired solution was divided into 2 reaction tubes. The first tube was added 3 drops of 2 N HCl which functioned as blank. The second tube solution was removed by one drop on a plate of drops, then added 3 drops of Dragendorff reagent. The orange deposits are formed indicating the presence of alkaloids (Widowati et al., 2016, 2017, 2018).

Steroids and Triterpenoids Test

Samples 10 mg in a plate of drops, plus glacial acetic acid until unmerged, left for 10-15 minutes then added one drop H2SO4, concentrated. If you produce a blue-green color, the sample contains a compound of steroid class, whereas if it produces a purple/red/orange color, the sample contains a triterpenoid class compound (Widowati et al., 2016, 2017, 2018).

Saponins Test

The basil leaves extract 10 mg dissolved using aquabidest in the reaction tube, then simmer in the bath for 5 minutes then filtered and then beaten strongly and added 1 N HCl. If the foam remains stable and still exists after being shed 1 N HCl Then the sample contains a saponin compound (Widowati et al., 2016, 2017, 2018).

Tannins Test

The sample of basil leaves extracts 10 mg dissolved in 2 ml of 2 N HCl in the test tube, then heated in 24 water baths for 30 minutes, then chill and then add amil Alcohol 500 μl. If the amil layer of alcohol is orange/red, the sample contains tannin compounds (Widowati et al., 2016, 2017, 2018).

Phenols Test

Dissolved extracts of basil leaves 10 mg in aquabidest as much as 5 ml, added solution FeCl3 1% as much as 500 μl. If the color is green/red/purple/blue/black, then the sample contains a group of compounds (Widowati et al., 2016, 2017, 2018).

Terpenoids Test

Samples as much as 10 mg into the drop plate added vanillin sufficiently, added H2SO4, concentrated one drop then homogenize. If it produces a purple color then the sample contains the terpenoid compounds (Widowati et al., 2016, 2017, 2018).
DPPH Scavenging Activity Assay

Antioxidant activity was tested by using the DPPH Scavenging method (2,2-diphenyl-2-picrylhydrazil). DPPH free radical compound is stable in aqueous or methanol solution and has a purple color shown by the absorption in solvent methanol at a wavelength of 515–520 nm. DPPH is sensitive to light, oxygen, and pH. However, it is stable in a radical form so that the possible measurement of antioxidant activity is quite accurate. Free radical DPPH can capture hydrogen atoms from the component extracts are mixed, then react into reduced shape and marked with the decline in the intensity of the color purple DPPH solution.

As many as 200 µL DPPH 0.077 mmol in the methanol added to 50 µL basil leaf ethanol extract sample on a microplate. The mix was incubated at room temperature for 30 minutes and then its absorbance values were measured at 517 nm wavelength using a microplate reader.

Negative controls were used to DPPH by as much as 250 µL, blank used as a DMSO absolute as much as 250 µL (Widowati et al., 2016, 2017, 2018).

DPPH Scavenging Activity (%) = \( 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100\% \)

\( A_{\text{sample}} = \text{Sample Absorbance}; \ A_{\text{control}} = \text{Negative Control Absorbance.} \)

Data Analysis

In this study, the analysis of the test used in this study is a one-way ANOVA (One-way ANOVA) followed by Post Hoc Tukey tests using a Test with a confidence level of 95% (\( \alpha = 0.05 \)).

RESULT AND DISCUSSION

Phytochemical Screening

The phytochemical screening is a qualitative analysis that includes diverse organic compounds formed and hoarded by living beings (Widowati et al., 2016, 2017, 2018). The results of phytochemical screening can be seen in Table I.

Table I shows the result that Basil leaves (Ocimum americanum L.) contains only 160 µL phosphate buffer and 20 µL sample. Next, mix the solution was added as many as 20 µL substrate l-dopa (1.5 mM) and incubated again at room temperature for 10 minutes. Absorbance is measured using a wavelength of 470 nm.

Inhibitory Activity (%) \( (\text{Gupta et al., 2009}) = \frac{C - S}{C} \times 100\% \)

\( C = \text{Absorbance of enzyme activity without the sample}; \ S = \text{Absorbance of enzyme activity with the addition of a tested sample.} \)

Data Analysis

In this study, the analysis of the test used in this study is a one-way ANOVA (One-way ANOVA) followed by Post Hoc Tukey tests using a Test with a confidence level of 95% (\( \alpha = 0.05 \)).
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The alkaloid test results show the formation of orange or orange colors. The alkaloid testing is carried out using a reagent/reactant of Dragendorff, where the resulting positive result is an orange precipitate for dragendorff. The deposits are potassium alkaloids. Reagent Dragendorff contains bismuth nitrate and potassium iodide in a solution of glacial acetic acid (potassium Tetraiodobismutat (III)). In the manufacture of Dragendorff reagent, the purpose of bismuth nitrate is dissolved in HCl in order not to occur hydrolysis reaction due to easily hydrolyzed salt-forming bismuth ions (BiO+) (Ergina, Nuryanti Sand Pursitasari, 2014).

The ingredients that are not contained in BLE, are tannins, triterpenoids, and terpenoids. However, the results of this research are similar to the research results of Ergina, Nuryanti S and Pursitasari, 2014 showing the unenlisted triterpenoid compounds that can be caused due by the use of solvents used in the extraction process are Polar and semi-polar solvents. Because the triterpenoid compound is a compound that is nonpolar so these compounds cannot be extracted perfectly on the solvent. Terpenoids compounds are generally nonpolar so that they can be extracted with non-polar n-hexane solvents (Ginting et al., 2017)

DPPH Scavenging Inhibitory Activity

2,2-diphenyl-2-picrylhydrazil (DPPH) is a compound of hydrogen radicals. DPPH will take a hydrogen atom in a compound. The reaction mechanism of the DPPH is taking place through electron transfer. The DPPH is purple solution gives the maximum absorption at 517 nm. DPPH solution will oxidize compounds in plant extracts. This process is characterized by the purple solution color fading away into yellow color (Widowati et al., 2016, 2017, 2018). The percentage DPPH scavenging activity of BLE and eugenol can be seen in Table II and the median inhibitory concentration (IC50) of samples based on DPPH scavenging activity can be seen in Table III.

Table II. DPPH Scavenging Activity of BLE and Eugenol

<table>
<thead>
<tr>
<th>Final Concentration (μg/mL)</th>
<th>Mean of DPPH Scavenging Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BLE</td>
</tr>
<tr>
<td>200</td>
<td>95.36 ± 1.54 e</td>
</tr>
<tr>
<td>100</td>
<td>94.83 ± 0.34 e</td>
</tr>
<tr>
<td>50</td>
<td>77.76 ± 5.20 d</td>
</tr>
<tr>
<td>25</td>
<td>57.26 ± 0.66 c</td>
</tr>
<tr>
<td>12.5</td>
<td>42.83 ± 0.14 b</td>
</tr>
<tr>
<td>6.250</td>
<td>34.50 ± 0.63 a</td>
</tr>
<tr>
<td>3.125</td>
<td>42.83 ± 0.14 b</td>
</tr>
<tr>
<td>1.563</td>
<td>46.32 ± 1.13 a</td>
</tr>
</tbody>
</table>

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05 (Tukey HSD post hoc test).
mechanisms. The main characteristic of antioxidants is the ability to capture or inhibit free radicals because these free radicals can oxidize nucleic acids, proteins, fats, or DNA and can initiate a degenerative disease (Fidrianny, Rizkiya and Ruslan, 2015).

Antioxidant activity test with the DPPH method is based on a reduction of DPPH which is a stable free radical. Free radical DPPH (2,2-Diphenyl-1-Picrylhydrazyl) has an electron that can provide maximum absorption at 517 nm (purple color). When antioxidants react with DPPH which is a stable free radical will be paired due to the presence of hydrogen donors (e.g. antioxidant) and transformed into DPPH-H later as a consequence of the absorption of DPPH will be reduced. In the form of DPPH-H, the result obtained is decolorization or color change to yellow due to electron capture. Thus, the more decolorization occurs, the higher the ability to reduce free radicals. In other words, antioxidants can reduce the purple DPPH radicals into yellow diphenyl-picrylhydrazine when the fluid from the DPPH is mixed with the substance that can donate hydrogen atoms, it will occur in the form of non-radical discoloration (Diphenyl-picrylhydrazine) with a loss of purple. (Shekhar and Goyal, 2014; Bamidele, Bamidele and Nnate, 2017)

**Table III. IC$_{50}$ Value of DPPH Scavenging Activity of BLE and Eugenol**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear Regression</th>
<th>R$^2$</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLE</td>
<td>y = 0.6403x + 37.73</td>
<td>0.88</td>
<td>19.16</td>
</tr>
<tr>
<td></td>
<td>y = 0.6236x + 36.762</td>
<td>0.95</td>
<td>21.23</td>
</tr>
<tr>
<td></td>
<td>y = 0.6202x + 36.808</td>
<td>0.94</td>
<td>21.27</td>
</tr>
<tr>
<td></td>
<td>y = 0.628x + 37.1</td>
<td>0.93</td>
<td>20.54</td>
</tr>
<tr>
<td>Eugenol</td>
<td>y = 0.4154x + 49.205</td>
<td>0.94</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td>y = 0.4176x + 49.094</td>
<td>0.95</td>
<td>2.17</td>
</tr>
<tr>
<td></td>
<td>y = 0.4262x + 48.959</td>
<td>0.94</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>y = 0.4262x + 48.959</td>
<td>0.94</td>
<td>2.44</td>
</tr>
</tbody>
</table>

Table IV. Antityrosinase Activity of BLE and Eugenol

<table>
<thead>
<tr>
<th>Final Concentration (µg/mL)</th>
<th>Mean of Antityrosinase Activity (%)</th>
<th>Ocimum americanum L.</th>
<th>Eugenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>67.98 ± 0.44 e</td>
<td>92.59 ± 1.49 f</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>54.89 ± 0.15 d</td>
<td>69.16 ± 0.21 e</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>48.11 ± 0.80 c</td>
<td>59.78 ± 0.26 d</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>45.62 ± 1.00 b</td>
<td>54.92 ± 0.89 c</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>39.06 ± 0.68 a</td>
<td>46.24 ± 0.29 b</td>
<td></td>
</tr>
<tr>
<td>6.25</td>
<td>37.55 ± 0.76 a</td>
<td>41.71 ± 0.39 a</td>
<td></td>
</tr>
</tbody>
</table>

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05 (Tukey HSD post hoc test).

**Antityrosinase Activity**

Whitening agents play a role in many levels of melanogenesis or melanin formation in the skin and little-known as a competitive inhibitor of tyrosinase. Tyrosinase enzyme is involved in the process of melanogenesis and catalysis of the oxidation process of tyrosine into dihydroxyphenylalanine (L-DOPA) and of L-DOPA into L-DOPA-quinone (Kamkaen, Mulri and Treesak, 2007; Wuttisin et al., 2017). Tyrosinase catalyzes the oxidation reactions that produce chromophore and tyrosine can be detected at wavelengths of up to 510 nm. The reaction of the enzyme tyrosinase substrate with L-DOPA is producing the orange color. The enzyme tyrosinase inhibitory activity was marked by a decline in orange color that is formed or the result of the reaction is colored more light. It also simultaneously mark the presence of antioxidant activity in the reaction (Fais et al., 2009; Tu and Tawata, 2015).

Based on Table IV, the antityrosinase activity of BLE and eugenol. In the highest concentration (200µg/mL), BLE has lower value (67.98 ±0.44 %) than eugenol does (92.59 ± 1.49 %).

Table V shows the result that BLE has higher IC$_{50}$ (35.59 ± 0.83 µg/mL) compared to eugenol.
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Table V. IC50 Value of Antityrosinase Activity of BLE and Eugenol

<table>
<thead>
<tr>
<th>Sampel</th>
<th>Persamaan</th>
<th>R²</th>
<th>IC50 (µg/mL)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLE</td>
<td>y = 0.4972x + 44.779</td>
<td>0.97</td>
<td>37.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>y = 0.4973x + 44.377</td>
<td>0.97</td>
<td>35.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>y = 0.4732x + 44.886</td>
<td>0.97</td>
<td>36.43</td>
<td>35.59 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>y = 0.4892x + 44.681</td>
<td>0.97</td>
<td>36.59</td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td>y = 0.2965x + 38.86</td>
<td>0.97</td>
<td>10.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>y = 0.3x + 39.243</td>
<td>0.95</td>
<td>11.31</td>
<td>10.87±0.41</td>
</tr>
<tr>
<td></td>
<td>y = 0.3013x + 39.023</td>
<td>0.97</td>
<td>10.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>y = 0.2993x + 39.05</td>
<td>0.97</td>
<td>10.50</td>
<td></td>
</tr>
</tbody>
</table>

(10.87±0.41 µg/mL). This indicates that BLE has an activity to inhibit tyrosinase enzyme, but has lower activity than eugenol does.

Hyperpigmentation is characterized by the absence of production and accumulation of melanin or an increase in the number of melanocyte cells. Accumulated hyperpigmentation can be caused by lentigo (flat brown spots on the skin), nevus (usually congenital nodules), and ephelis (red spots), or inflammatory states such as acne and eczema (Di Petrillo et al., 2016). Bleach agents play a role in various levels in melanogenesis or the formation of melanin in the skin and are widely known as competitive inhibitors of tyrosinase. Tyrosinase is an enzyme involved in the process of melanogenesis and the catalyzation of the process of oxidation of tyrosine into dihydroxyphenylalanine (DOPA) and from DOPA into DOPA-quinone.

CONCLUSION

Basil leaf extract has lower antioxidant activity through the DPPH scavenging activity and antiaging activity through the inhibition of tyrosinase compared to the eugenol compound. From phytochemical assay obtained that BLE contains flavonoids, saponins, phenols, steroids, and alkaloids as well as negative tannins, triterpenoids, and terpenoids. IC50 value of DPPH scavenging activity and tyrosinase inhibition of BLE indicates that BLE has potential as a natural antioxidant and antiaging ingredient for daily consumption.

ACKNOWLEDGEMENT

We gratefully acknowledge the methodology and facilities support of the Biomolecular and Biomedics Research Center, Aretha Medika Utama, Bandung, Indonesia. Thank you also to the Faculty of Medicine at Prima Indonesia University which has provided research facilities as well as the parties involved in this research.

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